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EFFECTS OF AGE ON SPERM SURVIVABILITY OF THE CAMEL SPERMATOZOA SUPPLEMENTED WITH GLYCINE BETAINE DURING STORAGE AT 5^{o}C.

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ABSTRACT

Two experiments were carried out. Twelve Fellahi camels were used in the present study and semen samples were collected using an artificial vagina.The camels were divided into three groups (4 each) according to their ages. The age of the first, second and third groups was <6 to 11, <11 to 16 and <16 to 21 years old, respectively. The first experiment aimed to define the effects of age on copulation time, semen characteristics and blood constituents in the male camel. The second experiment aimed to define the effects of glycine betaine (GB) addition to the cooled semen at levels of 100 and 200 mM on semen quality of the camel at <6 to 11 years, during storage at 5^{o}C for up to 3 days. Semen was diluted with lactose-yolk citrate (LYC) extender.The penetrating ability of spermatozoa supplemented with GB into she-camel cervical mucus, during incubation at 37^{o}C was recorded. The obtained results showed that copulation time (min) and semen-ejaculate volume (ml) were significantly (P<0.05) better in Fellahi camels at <6-11 and <11-16 years than <16-21 years. Semen colour was Creamy white, Creamy White and Milky white, whereas semen consistency was Viscous, Viscous and Semi-viscous of the camels at<6-11, <11-16 and<16-21 years, respectively. However,seminal pH value was insignificantly between different ages of the camels. The percentages of sperm motility and sperm-cell concentration (X10^{6}/ ml) were significantly (P<0.05) higher,while the percentages of dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of the camel spermatozoa at <6-11 and <11-16 years were significantly (P<0.05) lower than camels at <16-21 years. In addition, the effects of age on Total protein, Albumin and Globulin concentrations (gm/100 ml) in the blood serum of the male dromedary camels were not significant. While, Total cholesterol, Potassium, Calcium, phosphorus concentrations (mg/100 ml), Zinc concentration (µg/100 ml) and testosterone concentration (ng/100 ml) were significantly (P<0.05) increased of the camels at <6 to 11 and <11 to 16 years compared to those with the camels at <16 to 21 years. Furthermore, Sodium

concentration (mg/100 ml) and activity of Aspartate-aminotransaminase (AST) and Alanine-aminotransaminase (ALT) enzymes were significantly (P<0.05) higher of the camels at <16 to 21 years than camels at <6 to 11 and <11 to 16 years in the blood serum (Experiment 1). Percentages of sperm motility and storagability of spermatozoa were significantly(P<0.05) higher,while the percentage of dead spermatozoa,abnormal spermatozoa, acrosome damage andchromatin damage of the camel spermatozoaat<6-11yearsadded withGBwere significantly (P<0.05) higher with different storage times at 5^{o}C than free-GB medium. The prolongation of storage time at 5^{o}C was significantly (P<0.05) lower of the camel semen quality either with GB or without GB medium. The penetrating ability of spermatozoa into she-camel cervical mucus was significantly (P<0.05)better with GB than free-GB medium during incubation at 37^{o}C for 4 hours. The advancement of incubation time at 37^{o}Cwas significantly (P<0.05) lower of the penetration score for several incubation times either with or without GB addition (Experiment 2). In conclusion, copulation time, semen quality, blood constituents and penetration score were better in camels at <6-11years supplemented with G.B.

Keywords:

Dromedary camels, Semen quality, Blood, Storage time, Glycine betaine.

INTRODUCTION

The camel (*Camelus dromedarius*) is an important livestock species that can be uniquely adapted to hot and arid environments. There is an urgent need to increase food production resources in Africa and other arid countries to rectify the dramatic increasing in human population and food shortage. That could be achieved under the harsh condition through using camels.

Breeding activity in the male dromedary camels in nomadic herds starts at five to six years of age and continues until the age of fourteen to fifteen years old with some minor differences according to breed and geographical location **(El-Wishy, 1988).** Reproductive management of females parameters such as age at first service can influence conception rate, calving rate and inter-calving interval **(Khanna *et al.*, 1993).**

The blood components are the mirror that reflects the health condition of animals. So, the biochemical studies under different fluctuating climatic conditions are very important to clinicians in the field during interpretation of their findings. Minerals and trace elements has long been known to be important in animal nutrition as they may be dietary

essential and vital to enzyme processes of living cells or have some metabolic activity, bone formation and reproductive performance **Zeidan *et al.*, 2001 and Matter, 2019)** in the male dromedary camel. Artificial insemination a useful tool in animal production and the technique can enhance breeding programs and to improve productivity when used wisely. In camel, AI can be applied to improve genetics such as increasing milk, meat production and racing ability. Achievement of the high reproductive activity depends partially on the success of AI which in-turn depends on the quality of semen and its dilution and storage with minimum loss of fertilizing ability **(Wilson, 1984).** Generally, the alive spermatozoa can be prolonged for several days at chilled storage (2-5^{o}C). However, satisfactory fertility results are not always achieved after as little as one day of storage **(Murase *et al*., 1990 and Zeidan *et al*., 2001).** Glycine betaine (GB), called betaine is a quaternary amine, a trimethyl derivative of the amino acid glycine (N, N-dimethylglycine) which protects plants against salt stress **(Storey and Wyn Jones, 1977)** and modulates cellular responses to osmotic stress **(Petronini *et al.*,1992).** It has been shown that GB enhances the quality of cryopreserved ram spermatozoa **(Sanchez-Partida *et al.*, 1998)** but its effects on camel spermatozoa have not been studied. The present study aimed to investigate the effects of age on copulation time and semen characteristics of the male dromedary camels. Blood constituents in the dromedary camels were also recorded (Experiment 1). The effect of glycine betaine (GB) addition to the cooled camel spermatozoa at levels of 100 or 200 mM on semen quality during storage at 5^{o}C for up to three days were recorded (Experiment 2). The penetrating ability of spermatozoa into she-camel cervical mucus added with GB during incubation at 37^{o}C for up to four hours was also assessed.

MATERIAL AND METHODS

The experimental work was carried out at the Laboratory of Veterinary **Public Health, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt and Private Camel Farm, Belbies, Sh**arkiya Governorate, during the period from December, 2017 to April, 2018. Two experiments were carried out. The first experiment aimed to investigate the effects of different ages (<6-11, <11-16 and <16-21 years) of Fellahi camels *(Camelus dromedarious)* on copulation time, semen characteristics and blood constituents with different ages of camels. The second experiment aimed to define the effects of GB addition (100 and 200 mM/l00 ml) according to **Zhang *et al.* (2001)** to the cooled camel semen at <6-11 years on semen quality, during storage at 5^{o}C for up to 3 days. The penetrating ability of the camel spermatozoa with

different concentrations of GB (100 and 200 mM / 100 ml) into she-camel cervical mucus, during incubation at 37^{o}C for 4 hours was also assessed.

1.Materials:

1.1. Experimental animals.

Twelve Fellahi camels (<6-21 years old and 500 to 600 kg live body weight) were used in the present study. The camels were divided into three groups (4 each) according to their age as follows: from < 6 to 11 years, <11 to 16 years and <16 to 21 years for the first, second and third groups, respectively. All camels were in healthy conditions and clinically free form external and internal parasites with a sound history of fertility in the herd.

The age of animals was determined on the basis on dental formula according to **Wilson (1984)** as follow:

1. Temporary or milk teeth: These teeth in the camel were 22 in number, the dental formula s: $\frac{1\text{-}1}{3\text{-}3}$ $\frac{3\text{-}3}{2\text{-}2}$ $\frac{1\text{-}1}{1\text{-}1}$ =22

Incisors Canines Premolars:

2. Permanent teeth:

The permanent teeth number 34, the dental formula is written as:

$\frac{1\text{-}1}{3\text{-}3}$ $\frac{1\text{-}1}{1\text{-}1}$ $\frac{3\text{-}3}{2\text{-}2}$ $\frac{3\text{-}3}{3\text{-}3}$ =34

Incisors Canines Premolars Molars:

1.2. Feeding and management.

The rations offered to camels were calculated according to **Banerjee (1988).** Clean fresh water was offered freely to all camels. Camels were housed in a yard which was provided with common feeding trough and a concrete floor provided with common sheltered water trough. The camels could move freely in enclosed area.

2. Methods:

2.1. Camel semen collection.

Six ejaculates were collected from each camel with different ages between 0.8: 00 and 10:00 a.m. using an artificial vagina (AV). A modified artificial vagina (30 cm long and 5 cm internal diameter, IMV, France) as the method described by **Zeidan (2002)** during the breeding season according to **Abd El-Roouf *et al.*, (1975)**. The AV was filled with water at

50-55^{o}C. The temperature inside the inner liner was stabilized at 45-50^{o}C. The ejaculates were usually comes in fractions. Fresh camel semen that has a jelly-like consistency is left for liquefaction for about 30-60 minutes to make the sperm attained motility.

2.2. Copulation time (minutes).

Duration of copulation was measured from the time of penile intromission into the artificial vagina until withdrawal as the method described by **Bravo *et al.* (2000).**

2.3. Semen characteristics.

2.3.1. Semen colour.

Semen colour was determined by direct visual examination from the collecting tube **(Bravo *et al.*, 2000).**

2.3.2. Semen consistency:

Semen consistency was qualified as viscous when semen did not drop from a Pasteur pipette, semi-viscous when some semen dropped from the Pasteur pipette to glass slide and liquid when semen was fluid and dropped readily from the Pasteur pipette according to **Bravo *et al.* (2000).**

2.3.3. Semen-ejaculate volume (ml).

Semen-ejaculate volume was determined using a conical graduated tube.

2.3.4. Hydrogen-ion concentration (pH).

Seminal pH value was measured using universal indictor paper and standard commercial stains according to **Karras (1952).**

2.3.5. Sperm motility (%).

Generally, camel sperm motility (%) was detected as an oscillatory motion of the flagellum but not progressive due to the viscous materials according to **Campbell *et al.* (1956).** With regard to extended semen, the percentage of sperm motility was determined using one drop of the extended semen after each storage period. The drop of the extended semen was covered by a warmed cover slip and immediately examined using high power magnification (400x).

2.3.6. Storagability (%).

Storagability percentage of the cooled camel spermatozoa referred to the percentage of original motile spermatozoa still motile after 3 days of storage time at 5^{o}C as the method described by **Yassen and El-Kamash (1970).**

2.3.7. Dead spermatozoa (%).

The eosin/nigrosine staining procedure was carried out by dissolving 1.67gm eosin and 10 gm nigrosine in distilled water up to 100 ml according to **Hackett and Macpherson (1965).**

2.3.8. Abnormal spermatozoa (%).

The morphology of abnormal spermatozoa (%) was determined in the same smears prepared for live/dead spermatozoa ratio **(Watson, 1975).**

2.3.9. Acrosome damage of spermatozoa (%).

Assessment of the percentages of acrosome damage (%) of spermatozoa was done according to **Watson (1975).**

2.3.10. Chromatin damage of spermatozoa (%).

Toluidine blue staining was performed as previously described by **Erenpreiss *et al.* (2004).** Smears were fixed in ethanol-acetic acid glactial (3:1, v/v) for 1 min and 70% ethanol for 3 mins. Smears were hydrolyzed for 20 mins in 1 Mm HCL, rinsed in distilled water and air-dried. One droplet of 0.025% Toluidine blue in Mcllvaine buffer (sodium citrate-phosphate) pH 4.0 was placed over each smear and then cover slipped. Smears were evaluated with light microscope magnification (x1000).The percentage of chromatin damage was estimated by evaluating 300 sperm cells in each smear. Spermatozoa stained as green to light blue were considered to have normal chromatin, while those stained dark blue to violet were considered to have damaged chromatin.

2.3.11. Sperm-cell concentration ($x10^6$/ml).

The spermatozoa were counted using hemocytometer according to **Khan (1971).**

3. Semen extension:

Semen samples were collected, pooled and evaluated of the camel at < 6-11 years and then extended with lactose-yolk-citrate (LYC) extender (2.9 g sodium citrate dehydrate, 0.04 g citric acid anhydrous, 1.25 g lactose and 10 ml egg-yolk, per 100 ml distilled water, 500 I.U/ml Penicillin and 500 µg Streptomycin Sulphate) according to **Musa *et al.* (1992).** Semen extension was carried out by adding the appropriate volume of the semen slowly to the extender as the method described by **Salisbury *et al.* (1978**). The final extension rate was 1ml semen: 4 extender.

4. Chilling of semen at 5^oC:

The test tubes containing extended camel semen at < 6-11 years were placed in a 500 ml

beaker containing water at 30°C with a thermometer in order to facilitate periodic check of the temperature during cooling period. Another test tubes containing extended semen only were placed in the beaker to maintain the extended temperature similar to that of semen. The beaker was placed in a refrigerator and gradually cooled till their temperature reached to 5°C during a period of 1.5-2.0 hours. The cooled spermatozoa were kept at 5°C for up to 3 days. After each storage time (0, 1, 2 and 3 days), percentages of sperm motility, dead spermatozoa, abnormal spermatozoa, acrosome damage and chromatin damage of spermatozoa, were recorded.

5. Blood serum constituents:

Blood samples were collected pre-slaughter from jugular vein in the non-heparinized vacutainer tube for each camel in different ages and centrifuged for 15 minutes at 8000 RCF. Serum samples were taken weekly and stored at-20°C until analysis. Total protein, Albumin, Globulin, Total cholesterol, Sodium, Potassium, Calcium, phosphorus, Zinc and Testosterone concentrations and activity of AST and ALT enzymes were recorded. Total protein, albumin and total cholesterol concentrations were determined calorimetrically according to the method described by **Tietz (1982).** Globulin level was calculated by subtraction of albumin content from the total protein content. Testosterone hormone concentration (T_2) was determined by Radioimmunoassay Technique (RIA) of Coal-Ab-Cont Kits (Diagnostic Products Corporation-Los Anglos, USA) according to **Abraham (1977).** Sodium, Potassium, Calcium, and Phosphorus concentrations were determined calorimetrically according to **Tietz (1982).** Zinc concentration was determined using 5P9 atomic absorption Spectrophotometry (Pye Unicam) as the method described by **Willis (1960).**

6. Biochemical analysis in seminal plasma (U/10^6 spermatozoa):

After each storage times (0, 1, 2 and 3 days), the cooled semen was centrifuged for 15 minutes at 8000 R/P/MF. Seminal plasma was separated and stored at-20°C until assay of enzymes. Activity of aspartate aminotransaminase (AST) and alanine-aminotransaminase (ALT) enzymes (Spectroliv-UV Auto, LIU-2602, and Labomed, USA) were recorded according to the method described by **Reitman and Frankle (1957).**

7. Glycine betaine addition to the cooled camel semen:

Semen samples were collected, evaluated and diluted with LYC extends. The diluted spermatozoa were cooled slowly by keeping the containing tubes at room temperature and put in a 5°C. Three media were compared. The first medium was similar to a non-Glycine betaine

(Woko Pure Chemical Industries, Lte, Japan) and kept as a control medium. The second and third media were contained diluted cooled semen with 100 and 200 mM Glycine betaine, respectively according to the **Zhang *et al.* (2001)**. Assessment of storagability of spermatozoa was also determined during storage at 5^{o}C.

8. Sperm penetration (Score):

Sperm penetration into she-camel cervical mucus was assessed as the follows: Cervical mucus was obtained from she-camels at <6-11 years. A portion of the mucus was sucked into polyethylene sealed tubes with 2 mm internal diameter to provide a column of 6 cm length. Camel semen was collected and diluted with LYC extender as described by **Salisbury *et al.* (1978)** added with different concentrations of GB(100 and 200 mM) and then placed into 2ml cuvettes (1 ml each). The tubes containing the mucus were insured (open end) into the cuvettes containing the diluted semen with GB incubated at 37^{o}C for up to 4 hours. Sperm penetration was judged as the rank score as described by **Eskin *et al.* (1973) and Hanson *et al.* (1982).**

3. Statistical analysis:

Data were statistically analyzed by one-way and two-way design (ANOVA) using General Linear Model (GLA) procedure of SAS **(SAS, 2006).**Duncan's Multiple Range Test **(Duncan, 1955)** was used to detect significant differences among means. Percentage values were transformed to arc-sin values before being statistically analyzed. Penetration score and sperm storagability were analyzed using Chi-square test.

The following model in the first and second experiments was as follows:

The first experiment:

$Y_{ij}= \mu + A_i + e_j$

Y_{ij}= is the observed value of the dependent variable determined from a sample taken from each animal.

μ= is the overall mean.

A_i= is the fixed effect of age.

e_j= is the residual error.

The second experiment:

$Y_{ijk}= \mu + G_i + S_j + (G_i \times S_j) + e_{ijk}$

Y_{ijk}= is the observed value of the dependent variable determined from a sample taken from each animal.

μ= is the overall mean.

G_i= is the fixed effect of glycine betaine concentration.

S_j= is the fixed effect of storage time.

(G_i x S_j)= is the first – order interaction between glycine betaine and storage time.

e_{ijk}= is the residual error.

RESULTS AND DISCUSSION

The first experiment:

1. Copulation time (minutes):

Copulation time was significantly (P<0.05) longer of the dromedary camels at <6 to 11 and <11 to 16 years than <16 to 21 years old (Table 1). The longest (P<0.05) time of copulation was recorded of the male camels at <6 to 11 years and the shortest (P<0.05) time was recorded of the camels at <16 to 21 years of age. The aging process has an effect on the rate of reproduction rather than the rate of catabolism. It is generally considered that steroid hormone secretion by the testes is increased with the age until eleven years, consequently decreased in old camels **(Zeidan *et al.*, 2001)**.

2. Semen characteristics:

2.1. Semen colour.

Semen colour was Creamy white and Creamy white of the dromedary camels at < 6 to 11 and <11 to 16 years, respectively and Milky white at <16 to 21 years old (Table 1). **Ahmadi (2001)** found that, semen colour was yellowish white, creamiest white and milky white in the male camels at 3-5, 6-11 and 12-20 years of age, respectively. **Zeidan *et al.* (2001)** found also that, semen colour was yellowish white, creamy white and milky white of the dromedary camels at 2.5 to 5, over 5 to 10 and over 10 to 20 years of age, respectively. The different colour of semen with different ages may be due to different concentrations of spermatozoa and semen consistency **(Zeidan *et al.*, 2001).**

2.2. Semen consistency.

Semen consistency of the dromedary camels at <6 to 11 and <11 to 16 years was viscous, viscous and semi-viscous at <16 to 21 years old, respectively (Table 1). Similar trends were reported by **Zeidan *et al*. (2001)** who found that, semen consistency of the male dromedary camels is semi-viscous at 2.5 to 5 years of age and viscous at 5 to 10 and 10 to 20 years of age. Viscosity of semen is usually attributed to the presence of mucopolysaccharides **(Garnica *et al.*, 1993)** which can be secreted only from bulbourethral glands or the prostate gland.

2.3. Semen-ejaculate volume (ml).

Semen-ejaculate volume was significantly higher (P<0.05) of the dromedary camels at <6 to 11 years than <16 to 21 years old and insignificantly higher in camels at <6 to 11 years than <11 to 16 years of age (Table 1). The differences in semen-ejaculate volume between camels at < 6 to 11 and <11 to 16 years of ages were insignificant. Similar trends were recorded by **Zeidan *et al.* (2001) and Matter (2019)** of the dromedary camels.

2.4. Hydrogen-ion concentration (pH).

Seminal hydrogen-ion concentration (pH) value was insignificantly different in different ages of camels (Table 1). Seminal pH value of the camels at <6 to11 years was insignificantly lower than camels at <16 to 21 years of age. Similar trends were recorded by **Zeidan *et al.* (2001) and Matter (2019)** of the dromedary camels.

2.5. Percentage of sperm motility.

The percentage of sperm motility was significantly (P<0.05) higher of the dromedary camels at <6 to 11 and <11 to 16 years than <16 to 21 years old (Table 1). However, the percentage of sperm motility was insignificantly higher of the camels at <6 to 11 years than <11 to 16 years of age. The highest (P<0.05) value of the percentage of sperm motility was recorded of the male camels at <6 to 11 years and the lowest (P<0.05) value was recorded of the camels at <16 to 21 years of age. Similar trends were recorded by **Zeidan *et al.* (2001) and Matter (2019)** of the dromedary camels**.** The advancement of age revealed hypoactive Leydig cells which are considered to be testosterone hormone producing factor, so this reflected on semen characteristics produced by the aged animals **(Tingari *et al.*, 1993).**

2.6. Percentage of dead spermatozoa.

The percentage of dead spermatozoa was significantly (P<0.05) higher of the dromedary camels at <16 to 21 years than <6 to 11 and <11 to 16 years old. Similarly, the percentage of dead spermatozoa was significantly (P<0.05) higher of the camels at <11 to 16 years than <6 to 11 years of age (Table 1). The highest (P<0.05)value of the percentage of dead spermatozoa was recorded of the camels at <16 to 21 years and the lowest (P<0.05) value was recorded of the camels at<6 to 11 years of age. Similar trends were recorded by **Zeidan *et al.* (2001) andMatter (2019)** of the dromedary camels. Similarly,**Ahmadi (2001)** showed that,the highest value of the percentage of dead spermatozoa was recorded of the dromedary camels at 12 to 20 years and the lowest value was recorded at 6 to 11 years of age. These results may be attributed to that, the advancement of age which may cause

disturbance in spermatogenesis or destruction or even death of the camel spermatozoa **(Musa *et al.*, 1992).**

2.7. Percentage of abnormal spermatozoa.

The percentage of abnormal spermatozoa was significantly (P<0.05) higher of the dromedary camels at <16 to 21 years than <6 to 11 and <11 to 16 years old (Table 1).Similarly, the percentage of abnormal spermatozoa was significantly (P<0.05) higher of the camels at<11 to 16 years than <6 to 11 years of age.The highest (P<0.05) value of the percentage of abnormal spermatozoa was recorded of the camels at <16 to 21 years and the lowest (P<0.05) value was recorded at <6 to 11 years of age. **Ahmadi (2001)** showed that, the lowest value of the percentage of abnormal spermatozoa was recorded of the male dromedary camels at 6 to 11 years and the highest value was recorded of the camels at 3 to 5 years of age. **Hemeida *et al.* (1985)** reported that incidence of testicular degeneration increased by 10.9-15.0% of the camels at 4 to 15 years to 25% at 5 to 20 years of age and to 50% in senile (over 20 years) camels, consequently, sperm production rates was decline (32.2 - 92.4%).

2.8. Percentage of acrosome damage.

The percentage of acrosome damage of spermatozoa was significantly (P<0.05) higher of the dromedary camels at <16 to 21 years than <6 to 11 and <11 to 16 years old (Table 1). The highest (P<0.05) value of the percentage of acrosome damage was recorded of the camels at <16 to 21 years and the lowest (P<0.05) value was recorded of the camels at <6 to 11 years of age.Similar trends were reported by**Zeidan *et al.* (2001)**of the dromedary camel.

2.9. Percentage of chromatin damage.

Data presented in (Table 1) revealed that, the percentage of chromatin damage of spermatozoa of the dromedary camels were significantly (P<0.05) lower with camels at <6 to 11 years than <11 to 16 and < 16 to 21 years. Similarly, the percentage of chromatin damage of spermatozoa was significantly (P<0.05) lower of the camels at <6-11 years than at 11-16 years of the dromedary camel spermatozoa (**El-Mahdy, 2019)**. However, **Pradana *et al*. (2016)** found that, the Friesians bull sperm chromatin integrity was not significantly different during storage at 5^{o}C. This phenomenon may be due to the decrease of adenosine triphosphate which activated apparently ability of resynthesizing. This was accompanied with precipitation fall in the rate of fructolysis, consequently increased chromatin damage **(Mann and Lutwak-Mann, 1981).**

2.10. Sperm-cell concentration (x10^6/ml).

Sperm-cell concentration was significantly (P<0.05) higher of the dromedary camels at <6 to 11 and <11 to 16 years than <16 to 21 years old (Table 1). However, sperm-cell concentration was insignificantly higher of the camels at <6 to 11 years than <11 to 16 years of age. Similar trends were recorded by **Zeidan *et al.* (2001) and Matter (2019)** of the dromedary camels.

Table (1): Effect of different ages on copulation time (mins) and semen characteristics of the dromedary camels (Means±SE).

Items	Age (years)		
	< 6 – 11	< 11 – 16	< 16 – 21
Copulation time (mins)	6.84±0.09[a]	6.38 ± 0.08[a]	4.61 ± 0.08[b]
Semen colour	Creamy white	Creamy white	Milky white
Semen consistency	Viscous	Viscous	Semi-visocous
Semen-ejaculate volume (ml)	6.40±0.90[a]	6.11±0.90[a]	4.23±0.07[b]
Hydrogen-ion-conc. (pH)	7.51±0.12[a]	7.65±0.12[a]	7.82±0.13[a]
Sperm motility (%)	69.18±1.74[a]	67.43±1.53[a]	58.91±1.34[b]
Dead spermatozoa (%)	23.01±0.18[c]	26.38±0.20[b]	32.46±0.26[a]
Abnormal spermatozoa (%)	14.25±0.11[c]	18.32±0.13[b]	24.62±0.19[a]
Acrosome damage (%)	3.12±0.08[c]	6.17±0.10[b]	8.03±0.15[a]
Chromatin damage (%)	1.46±0.05[c]	3.80±0.08[b]	5.18±0.13[a]
Sperm-cell conc. (x10^6/ml)	385.91±9.12[a]	374.85±8.90[a]	312.53±6.42[b]

a-c: Within rows, within ages, means with different superscripts letters differ significantly (P<0.05).

3. Blood serum constituents:

3.1. Total protein, albumin and globulin concentrations (gm/dI).

Data presented in Table 2 showed that total protein, albumin and globulin concentrations (gm/dI) in the blood serum were not significant with different ages of the male dromedary camels. The decrease of total protein with the progress of age may be attributed to low semen characteristics and adaptability of camels which represented the potent stimulus of semen quality. Similar trends were reported by **Ahmadi (2001)** in the male dromedary camels.

3.2. Total cholesterol and testosterone concentrations.

Total cholesterol (mg/dI) and testosterone concentrations (mg/ml) in the blood serum of the male dromedary were significantly (P<0.05) increased of the camels at <6-11 and <11-16 compared to those with <16-21 years (Table 2). The highest (P<0.05) values of the total cholesterol and testosterone concentrations in the blood serum of the male dromedary camels were recorded with <6-11, while the lowest (P<0.05) values were recorded in the camels at <16-21.

Abd El-Azim (1996) confirmed that, the highest value of cholesterol concentration was recorded of the male camels at 5 to 10 years. **Khan and Kohli (1973)** showed higher values in cholesterol concentration of the male camels before and during rut. It was ranged from 49.8 to 85.3 mg/100 ml of with a mean value of 65.58 mg/100 ml. Moreover, testosterone blood serum was significantly higher which is parallel to increase of sexual activity of the male dromedary camels. This reflect the fact that, the high androgen of the male camel with the direct cause of the characteristics of its sexual behavior of the camels at <6-11 and <11-16 compared to those with camels at <16-21 years. These results are partially agreement with those of **Bedrak *et al.* (1983) and Zeidan *et al.* (2001)** of the male dromedary camel.

3.3. Mineral concentrations (mg/100ml).

Sodium concentration (mg/100 ml) in the blood serum of the male dromedary camels was significantly (P<0.05) increased of the camels at <16-21 compared to those with <6-11 and <11-16 years(Table 2). The highest (P<0.05) value of blood sodium concentration of the male dromedary camels was recorded with camels at <16-21. While, the lowest (P<0.05) value was recorded with <6-11 and<11-16 years.These results may be attributed to the combined effects of both absorption and reabsorption of sodium and chloride from the alimentary tract and Kidney, under the effect of aldosterone hormone which had higher levels with the camels at <6-11 and <11-16 years and this was accompanied by increase of plasma sodium level **(Yagil and Etzion,1979 andAbd El-Azim,1996)** in the dromedary camels**.** However, Potassium, Calcium and phosphorous concentrations (mg/100 ml) and Zinc concentrations (µg/100 ml) in the blood serum of the male camels were significantly (P<0.05) higher of the camelsat<6-11 and <11-16than<16-21 years(Table 2).Thedecreasein potassiumconcentration in the camels at <16-21 years may be due to lower semen characteristics of the male dromedary camels. Similarly,the increaseofCalcium,Total phosphorus and Zinc concentrations of the camels may be high feed efficiency and metabolic rate consequently high semen quality of the camels at <6 to 11 and <11to16 years compared to those camels at <16 to 21 years (Table 2). Similar findings were reported by **Zeidan and Abbas (2004) and Amin *et al.* (2007)** of the male dromedary camel.

Table (2): Effects of different ages on blood serum constituents of the male dromedary camels(Means±SE).

Items	Age (year)		
	< 6 -11	< 11 – 16	< 16 - 21
Total protein (gm/100ml)	7.53±0.18[a]	7.06±0.17[a]	6.94±0.14[a]
Albumin (gm/100ml)	3.72±0.18[a]	3.61±0.16[a]	3.69±0.15[a]
Globulin (gm/100ml)	3.81±0.16[a]	3.45±0.14[a]	3.25±0.13[a]
Total cholesterol (mg/100ml)	86.17±1.34[a]	85.11±1.32[a]	74.62±1.20[b]
Sodium (meg/100ml)	138.19±4.06[b]	140.12±4.15[b]	163.18±4.25[a]
Potassium (meg/100ml)	14.08±0.26[a]	13.91±0.24[a]	10.72±0.19[b]
Calcium (mg/100ml)	12.74±0.23[a]	12.36±0.21[a]	8.14±0.16[b]
Phosphorus (mg/100ml)	10.16±0.18[a]	10.12±0.15[a]	7.18±0.14[b]
Zinc (µg/100ml)	125.18±3.72[a]	123.29±3.94[a]	112.53±2.58[b]
Aspartate-aminotransaminase (U/L)	32.61±0.72[b]	32.64±0.75[b]	41.65±0.64[a]
Alanine- aminotransaminase (U/L)	46.11±0.91[b]	51.73±1.12[b]	57.18±1.26[a]
Testosterone concentration (ng/ml)	4.76±0.12[a]	4.12±0.10[a]	2.49±0.08[b]

a-c: Within a rows, within semen quality, means with different superscripts letters differ significantly (P<0.05).

3.4. Enzymatic activity (U/L).

Activity of AST and ALT (U/L) enzymes in blood serum of the male dromedary camels was significantly (P<0.05) higher in the camel having Poor quality of semen than Normal and Good quality of semen (Table 2).Similarly, the activity of the blood serum AST and ALT enzymes was significantly (P<0.05) increased of the camels having Normal semen quality compared to those with Good semen quality (Table 2). In general, the blood enzymes are easily and often influenced by the external condition including feeding practices, type of shelter and many other aspects of the herd management, since they are ultimately related to metabolism especially environmental ones when measuring the enzyme activity in any animal the adaptability of the camels having Normal and Good semen quality was significantly better than camels with Poor semen quality.Similar trends were recorded by **Zeidan and Abbas (2004) and Matter (2019).**

The second experiment:

1. Percentage of motile camel spermatozoa:

Supplementation of GB at concentrations of 100 and 200 mM to the extended cooled semen with LYC extender increased significantly (P<0.05) the percentage of motility and storagability of the camel spermatozoa as compared to free-GB medium during storage at 5°C for up to 3 days (Table 3). Similar trends were recorded by **Sanchez-Partida *et al*. (1992)** in ram and **Zhang *et al.*(2001)** in Friesian bull semen. Betaine is a member of the family of compounds known as compatible solutes and bacteria, as well as mammalian kidney cells use specific betaine transports to internalize the molecule, particularly in hyperosmotic environments **(Nakanishi *et al.*, 1990).**Once inside the cell compatible solutes help to regulate internal osmolality with minimal deleterious effects on other cell functions, such as enzymes **(Petronini *et al.*,1992).** It has been shown that GB can preserve the three-dimensional structureof complexmolecules such as RNAse subjected tothermal destabilization in the presence of urea possibly through hydration interactions **(Burg and Peters,1998).**The prolongation of storage time at 5°C decreased significantly (P<0.05) the percentage of motile camel spermatozoa at all different concentrations of GB or free-GB medium. It is of interest to note that,the percentage of motility of the cooled camel spermatozoa increased significantly (P<0.05) during the first day and then decreased significantly (P<0.05) as time of storage increase (Table 3).These results are in agreement with those of**Zhang *et al.*(2001)** in bull spermatozoa.

Table (3): Mean percentages of motile camel spermatozoa supplemented with glycine betaine; during storage at 5°C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)			Mean
	Control	100	200	
0	64.52±0.86	68.74±1.16	68.15±1.04	67.13±1.03^{A}
1	56.14±0.67	64.82±1.02	64.73±0.91	61.89±1.02^{B}
2	45.23±0.60	58.64±0.84	57.42±0.73	53.76±0.65^{C}
3	23.16±0.41	47.11±0.70	45.67±0.68	38.64±0.28^{D}
Mean	47.26±0.73^{b}	59.82±0.93^{a}	58.99±0.86^{a}	55.35
Storagability (%)	35.89^{b}	68.53^{a}	67.0.1^{a}	57.55

A-D Values with different superscripts within a column are significantly different (P<0.05).

a-b Values with different superscripts within a row are significantly different (P<0.05).

2. Percentage of dead camel spermatozoa:

Supplementation of GB at concentrations of 100 and 200mM to the extended cooled semen with LYC extender decreased significantly (P<0.05) the percentage of dead camel spermatozoa as compared to free-GB medium, during storage at 5^{o}C for 3 days (Table 4). The lowest (P<0.05) value of the percentage of dead spermatozoa was recorded with the cooled camel semen added with 100mM and the highest (P<0.05) value was recorded with free-GB medium. Similar findings were recorded by **Zhang *et al.* (2001)** in bull spermatozoa.

It is of interest to note that, the prolongation of storage time at 5^{o}C increased significantly (P<0.05) the percentage of dead camel spermatozoa at all different concentrations of GB addition or free-GB medium. The percentage of dead spermatozoa increased significantly (P<0.05) as time of storage increase (Table 4). **Shannon and Curson (1972)** found that dead spermatozoa were a source of amino acid oxidase which cause the production of H_2O_2, consequently, increased the percentage of dead spermatozoa as the time of storage increase. Similar trend was reported by **Zeidan (2002)** of the dromedary camel spermatozoa.

Table (4): Mean percentages of dead camel spermatozoa supplemented with glycine betaine, during storage at 5^{o}C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)			Mean
	Control	100	200	
0	30.63±0.17	23.45±0.11	24.15±0.10	26.07±0.14^{D}
1	37.81±0.28	28.64±0.16	29.42±0.18	31.95±0.20^{C}
2	46.72±0.29	34.16±0.20	37.50±0.23	39.46±0.46^{B}
3	68.45±1.03	46.84±0.38	49.36±0.35	54.88±0.61^{A}
Overall mean	45.90±0.34^{a}	33.27±0.26^{b}	35.10±0.28^{b}	38.09

A-D Values with different superscripts within a column are significantly different (P<0.05).

a-b Values with different superscripts within a row are significantly different (P<0.05).

3. Percentage of abnormal camel spermatozoa:

Addition of GB at concentrations of 100 and 200 mM to the diluted cooled semen with LYC extender decreased significantly (P<0.05) the percentage of abnormal camel spermatozoa as compared to free-GB medium, during storage at 5^{o}C for 3 days (Table 5).The lowest (P<0.05) value of the percentage of abnormal spermatozoa was recorded with 100 mM GB and the highest value (P<0.05) was recorded with free-GB medium. It was a very potent and efficient

endogenous radical scavenger. It reacted with the highly toxic hydroxyl radical and provides protection against oxidative damage to biomolecules. These results are in agreement with those recorded by **Zhang *et al.*(2001)**of bull spermatozoa.

Table (5): Mean percentages of abnormal camel spermatozoa supplemented with glycine betaine; during storage at 5^{o}C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)			Mean
	Control	100	200	
0	17.32±0.16	15.19±0.11	15.84±0.12	16.11±0.13^{D}
1	22.41±0.35	19.16±0.15	20.08±0.16	20.55±0.17^{C}
2	28.67±0.35	23.72±0.19	25.41±0.32	25.93±0.19^{B}
3	45.25±0.63	32.61±0.54	34.10±0.53	37.32±0.46^{A}
Overall mean	28.41±0.43^{a}	22.61±0.36^{b}	23.85±0.21^{b}	24.97

A-D Values with different superscripts within a column are significantly different (P<0.05).

a-b Values with different superscripts within a row are significantly different (P<0.05).

The prolongation of storage time at 5^{o}C increased significantly (P<0.05) the percentage of abnormal camel spermatozoa at all different concentrations with GB addition or free-GB medium (Table 5). These results are in agreement with those of **Zhang *et al.* (2001)** in bull spermatozoa.

4. Percentage of acrosome damage of spermatozoa:

The percentage of acrosome damage of camel spermatozoa as affected by GB addition was significant (P<0.05). The highest (P<0.05) value of the percentage of acrosome damage of spermatozoa was recorded with free-GB medium, while the lowest (P<0.05) value was recorded at 100 mM GB (Table 6).

Jones and Stewart (1979) indicated that extension and cooling of bull semen to 5^{o}C caused acrosome swelling in about 50% of the spermatozoa. Subsequent freezing and thawing caused considerable ultrastructural changes to the acrosomes (disruption of the plasma and outer acrosome membranes and dispersion of the acrosomal contents) and middle pieces (breakage of the plasma membrane and a reduction in the electron density of the mitochondrial matrix) of a high proportion of spermatozoa.

Similar trends were recorded by **Matter (2019)** of the dromedary camel spermatozoa. Moreover, storage of semen at low temperatures caused structural damage as a result of the cold shock. The changes involved damage to the plasma membrane over the acrosome and the outer acrosome membrane and damage to the plasma membrane of the middle piece.

These changes are followed by a decrease in the proportion of spermatozoa with intact acrosomes and an increase in the release of enzymes into the extracellular medium. Therefore, the morphological characteristics of sperm acrosomes and enzyme concentrations in the extracellular medium with initial motility gives the best indication so far of initial quality, especially for frozen bull semen **(Zeidan *et al.*, 1998).**

The prolongation of storage time at 5^{o}C increased significantly (P<0.05) the percentage of acrosome damage of the cooled camel spermatozoa at all different concentrations of GB or free-GB medium (Table 6). These results are in agreement with those of **Zhang *et al.* (2001)** of bull spermatozoa.

Table (6): Mean percentages of acrosome damage of the camel spermatozoa supplemented with glycine betaine; during storage at 5^{o}C for up to 3 days (Mean ± SE).

Storage time (day)	Glycine betaine concentration (mM)			Mean
	Control	100	200	
0	7.15±0.11	4.23±0.08	4.72±0.08	5.36±0.09^{C}
1	8.34±0.16	4.68±0.0	4.81±0.09	5.94±0.10^{C}
2	11.61±0.19	5.14±0.11	5.46.0.10	7.40±0.12^{B}
3	14.18±0.23	6.87±0.13	7.58±0.12	9.54±0.21^{A}
Overall mean	10.32±0.18^{a}	5.23±0.11^{b}	5.64±0.10^{b}	7.06

A-C Values with different superscripts within a column are significantly different (P<0.05).

a-b Values with different superscripts within a row are significantly different (P<0.05).

5. Percentage of chromatin damage (%):

The percentage of chromatin damage of the camel spermatozoa added with 100 and 200 mM of GB was significantly (P<0.05) lower as compared to free-GB medium, during storage at 5^{o}C for up to 3 days (Table 7). Similar trends were recorded by **Zeidan *et al.* (2001) and Matter (2019)** in the dromedary camel spermatozoa.

There were many fluctuations in damage of DNA spermatozoa such as imperfect of spermatogenesis process; apoptosis, reactive oxygen species, in vitro handling, and type of extender and cryopreservation stress **(Baiee *et al*., 2017). Lioyd *et al.* (2012)** confirmed that sperm DNA integrity was better in commercial diluent could be significantly increased DNA fragmentation during storage at 5^{o}C for 48 hours. Similar trends were recorded by **El-Mahdy (2019)** of the dromedary camel spermatozoa.

It is of interest to note that,the prolongation of storage time at 5°C for 3 days was significantly (P<0.05) increased the percentage of chromatin damage of the camel spermatozoa in different concentrations of GB or free-GB medium (Table 6). These results are in agreement with those of **El-Mahdy (2019)** of the dromedary camel spermatozoa.

Table (7): Mean percentages of chromatin damage of the camel spermatozoa supplemented with glycine betaine, during storage at 5°C for up to 3 days.

Storage time (day)	Glycine betaine concentration (mM)			Mean
	Control	100	200	
0	4.60±0.08	3.19±0.06	3.46±0.05	3.75±0.05^{C}
1	5.24±0.11	3.57±0.07	3.81±0.06	4.20±0.07^{C}
2	6.32±0.12	4.32±0.08	4.65±0.08	5.09±0.13^{B}
3	10.18±0.16	6.11±0.13	7.58±0.12	7.95±0.14^{A}
Overall mean	6.58±0.13^{a}	4.29±0.09^{b}	4.87±0.08^{b}	5.24

A-C Values with different superscripts within a column are significantly different (P<0.05).
a-b Values with different superscripts within a row are significantly different (P<0.05).

6. Sperm penetration into cervical mucus:

Fig. (1) showed that, the penetrating ability of spermatozoa into she-camel cervical mucus was significantly (P<0.05) better with GB addition than free-GB medium. However, the advancement of incubation time at 37°C for up to 4 hours was significantly (P<0.05) decreased the penetrating ability of spermatozoa into she-camel cervical mucus with different concentrations of GB or free-GB medium.

Aitken *et al.* (1983) found a close correlation between human movement of spermatozoa and their penetrating ability into cervical mucus. **Murase *et al.* (1990)** reported that, the duration of sperm motility and penetration distance in the mucus closely correlated to the pregnancy and conception rate. Similar findings were recorded by **Zeidan (2002) and El-Mahdy (2019)** of the dromedary camels.

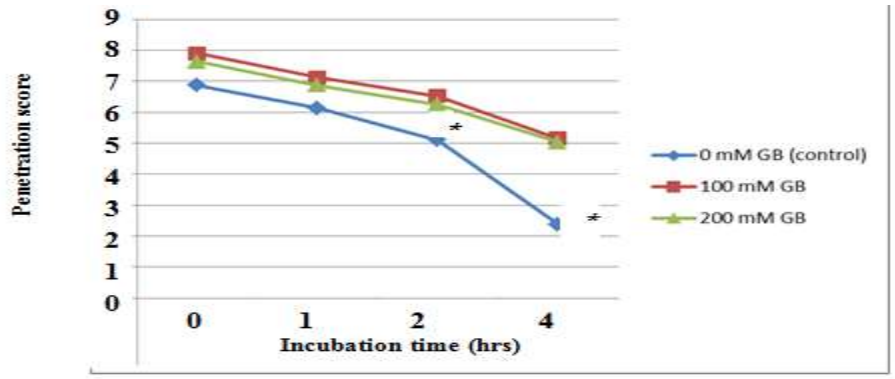

Fig. (1): Penetrating ability of the dromedary camel spermatozoa into she-camel cervical mucus with different glycine betaine (GB) concentrations during incubation at 37ºC for 4 hours.

* Significantly different from control in the same time point (P<0.05).

In conclusion, the male dromedary camels at < 6-11 and <11-16 years of age showed better copulation time, semen characteristics and blood constituents than camels at < 16 - 21 years. Camel semen quality and penetrating ability of spermatozoa into she-camel cervical mucus are enhanced by addition of GB during storage.

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تأثير العمر على بقاء وحيوية الحيوانات المنوية للجمال مع إضافة الجليسين بيتان عند الحفظ على درجة حرارة 5م°

السيد أبو الفتوح أحمدي

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الملخص العربي

أجريت هذه الدراسة على عدد 12 ذكر جمل فلاحي في تجربتين وتم جمع السائل المنوي منهم بإستخدام المهبل الاصطناعي وقد قسمت الجمال على حسب أعمارها إلى ثلاثة مجاميع (4 لكل مجموعة) هـي أكبر من 6 to 11 > ، 11 to 16 > و 16 to 21 > سنه على التوالي. وكانت التجربة الأولى تهدف إلى معرفة تأثير العمر على فترة الجماع، صفات السائل المنوي وكذا مكونات الدم. أما التجربة الثانية فقد كانت تهدف إلى معرفة تأثير إضافة الجليسين بيتان (GB) على نوعية السائل المنوي للجمال عن عمر أكبر من 6 حتى 11 سنه أثناء الحفظ على درجة حرارة 5°م لمدة 3 أيام. تم تخفيف السائل المنوي بمخفف اللاكتوز - سترات (LYC). كذلك تم معرفة مدى نفاذية الحيوانات المنوية داخل مخاط عنق الرحم أثناء التحضين على درجة حرارة 37°م المضاف إليها GB.

اوضحت النتائج ان هناك تحسن معنوي (على مستوى 0.05) في فترة الجماع (دقائق) وحجم قذفة السائل المنوي (مل) في الجمال الفلاحي عن عمر أكبر من 6 حتى 11 ، أكبر من 11 حتى 16 سنه عن الجمال عن عمر أكبر من 16 حتى 21 سنه من العمر. كان لون السائل المنوي ابيض كريمي ، ابيض كريمي وابيض بلون اللبن ، في حين كانت كثافة السائل المنوي لزجة ، لزجة وشبه لزجة في الجمال عن عمر أكبر من 6 حتى 11 ، أكبر من 11 حتى 16 سنه ومن 16 حتى 21 سنه على التوالي. في حين كانت قيمة الأس الهيدروجين (pH) غير معنوية بين الاعمار المختلفة للجمال. زيادة النسبة المئوية لحركة الحيوانات المنوية وتركيزها (× 106/مل) بدرجة معنوية (على مستوى 0.05) ، بينما انخفضت النسبة المئوية للحيوانات المنوية الميتة والشاذة وشذوذ الأكروسوم وشذوذ الكروماتين في الحيوانات المنوية للجمال عن عمر أكبر من 6 حتى 11 وأكبر من 11 حتى 16 سنه عن الجمال عن الجمال عند عمر أكبر من 16 حتى 11 سنه وأكبر من 11 حتى 16 سنه عن الجمال عن عمر أكبر من 16 حتى 21 سنة. هذا بالإضافة إلى أن تأثير العمر على تركيز كلا من البروتين الكلي ، الالبيومين والجلوبيولين (gm/100 ml) في سيرم دم ذكور الجمال العربية كان غير معنوي. بينما كان هناك زيادة معنوية (على مستوى 0.05) في سيرم دم الجمال العربية في كلاً من الكوليسترول الكلي (meg/100 ml) والبوتاسيوم (mg/100 ml) والكالسيوم (mg/100 ml) والفوسفور (mg/100 ml) والزنك (µg/100 ml) والتستسترون (ng/100 ml) في سيرم دم الجمال العربية مقارنة بالجمال عن عمر أكبر 16 حتى 21 سنة . كما كانت هناك زيادة معنوية (على مستوى 0.05) في تركيز الصوديوم (meg/100ml) ونشاط إنزيم ALT , AST في الجمال العربية عند عمر أكبر من 16 حتى 21 سنه عن الجمال عن عمر أكبر من 6 حتى 11 وأكبر من 11 إلى 16 سنة (التجربة الأولى). زيادة النسبة المئوية لحركة الحيوانات المنوية وقدرتها على الحفظ معنويا (على مستوى 0.05) مع انخفاض النسبة المئوية للحيوانات المنوية الميتة والشاذة وشذوذ الاكروسوم وشذوذ الكروماتين بدرجة معنوية (على مستوى 0.05) في الحيوانات المنوية للجمال عن عمر أكبر من 6 إلى 11 سنه مع إضافة (GB) بيتان عن الخالية من (GB) وذلك في فترات الحفظ المختلفة على درجة حرارة 5م°. إنخفاض نوعية الحيوانات المنوية مع التقدم في فترات الحفظ على درجة حرارة 5°م سواء بدون أو مع إضافة GB. زيادة معدل نفاذية الحيوانات المنوية معنوياً (على مستوى 0.05) داخل مخاط عنق الرحم للحيوانات المنوية المضاف إليها (GB) عن الخالية من الـ (GB) اثناء فترة التحضين على درجة حرارة 37°م لمدة أربع ساعات مع إنخفاض معنوي (على مستوى 0.05) في معدل النفاذية مع التقدم في فترات التحضين المختلفة (التجربة الثانية).